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Cosmocercoides variabilis (Nematoda: Cosmocercoidea) Populations in the Eastern American Toad, *Bufo a. americanus* (Salienta: Bufonidae), from Western West Virginia

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ABSTRACT: *Cosmocercoides variabilis* was recovered from the small and large intestines of *Bufo a. americanus* in western West Virginia. Toads were collected from 2 different sites during the April breeding seasons of 1994 and 1995 (Cabell Co.) and 1993 and 1995 (Wayne Co.). Prevalence was 100% in both host sexes from Cabell Co. (25 of 25 females; 48 of 48 males). Prevalences in Wayne Co. toads were 87.5% for females (14 of 16) and 57.1% for males (28 of 49). A total of 3,114 *C. variabilis* were collected: 2,878 from 73 Cabell Co. hosts and 236 from 42 Wayne Co. hosts. The 3,114 nematodes were distributed as 1,852 females, 1,196 males, and 66 juveniles. Specific mean intensities, and numbers and sex of nematodes collected by host sex and collection site, are reported.

KEY WORDS: *Cosmocercoides*, *Bufo*, nematode sex ratios, West Virginia.

Cosmocercoides variabilis (Harwood, 1930) Travassos, 1931, a nematode parasite of toads, was considered a synonym of the molluscan parasite *C. dukae* (Holl, 1928) Travassos, 1931, by Ogren (1953, 1959), who presumed that amphibians acquired *C. dukae* infections by ingesting infected molluscs. More recently, however, Vanderburgh and Anderson (1987a) demonstrated that these 2 species of *Cosmocercoides* are distinct and that *C. variabilis* is an amphibian parasite. There are few data on infections of *C. variabilis* in natural toad populations and no data on this nematode species from toad populations in West Virginia. The purpose of this study was to investigate *C. variabilis* infections in breeding populations of *Bufo a. americanus* Holbrook, 1836, in 2 western West Virginia locations.

Materials and Methods

Sample areas and procedures

West Virginia collection sites, separated by a distance of 35 km, were located in Cabell Co. (Green Bottom Wildlife Management Area, 38°35′11″ N, 82°15′39″ W, elevation 550 ft) and Wayne Co. (Beech Fork Lake Lower Bowen Campground, 38°18′19″N, 82°20′49″W, elevation 600 ft). Toads were collected by hand during the April breeding seasons of 1994 and 1995 at the former site and 1993 and 1995 at the latter site. Host sample sizes by sex, site, and year of collection are given in Table 1. Toads were brought to the laboratory and necropsied within 24 hr of capture. Prior to necropsy, each toad was weighed to the nearest 0.1 g. Each toad was killed by pithing, and the small and large intestines were removed for examination. Every *Cosmocercoides variabilis* encountered in a toad was kept, sexed with a stereomicroscope, and counted. In those few instances where sex was questionable, the worms were cleared in lactophenol and sexed using a compound microscope. When sex could not be determined, the individual was considered a juvenile. Voucher specimens of *Cosmocercoides variabilis* have been deposited in the U.S. National Parasite Collection, Beltsville, Maryland, under accession numbers USNPC No. 85430 (male) and USNPC No. 85431 (female), respectively. References to prevalence and mean intensity follow the definitions of Margolis et al. (1982).

Statistical analyses

Toad weights, recorded by sex and collection site, were compared by an unpaired, 2-tailed Student's *t*-test. Data on mean *C. variabilis* intensities were analyzed for statistical significance by the Wilcoxon rank-sums test using a statistical computer package (SAS Institute Inc., 1989; NPAR1WAY, SAS Institute, Cary, North Carolina). Nematode sex ratios were compared by a chi-square test. Levels of statistical significance for each test are shown in the appropriate tables.

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Table 1. Sample sizes and mean weights, by sex, for *Bufo a. americanus* collected at Beech Fork (BF) and Green Bottom (GB), West Virginia. Toad weights for both collection years at each site combined for statistical comparisons.

Site	Year	Sample size ♀♀	Sample size ♂♂	Mean (±1 SD) toad weight in grams ♀♀	Mean (±1 SD) toad weight in grams ♂♂
BF	1993	9	34	59.9 (19.1)	34.8 (6.5)
	1995	7	15	58.9 (11.5)	30.6 (5.9)
BF	Combined	16	49	59.5 (15.7)*‡	33.5 (6.4)*§
GB	1994	22	28	44.9 (10.0)	29.0 (5.6)
	1995	3	20	49.4 (8.4)	29.2 (5.2)
GB	Combined	25	48	45.5 (9.8)†‡	29.1 (5.5)†§

* BF female weight vs. male weight: $t = 9.49$; 63 df; $P < 0.001$.

† GB female weight vs. male weight: $t = 9.18$; 71 df; $P < 0.001$.

‡ BF female weight vs. GB female weight: $t = 3.52$; 39 df; $P < 0.05$.

§ BF male weight vs. GB male weight: $t = 3.57$; 95 df; $P < 0.001$.

Table 2. Two-way comparisons of mean intensities for *Cosmocercoides variabilis* infections in *Bufo a. americanus* calculated by the Wilcoxon ranked-sums test. Comparisons 1 and 2 are for the same host sex between sites, whereas comparisons 3 and 4 are for different host sexes within sites. BF = Beech Fork; GB = Green Bottom; $\bar{x}$ = mean intensity; Obs. = Σ observed ranked scores; Exp. = Σ expected ranked scores under the null hypothesis that distribution of nematodes in the 2 populations being compared are similar. H_0 rejected if $P > 0.05$.

	Site	Host sex (*n*)	$\bar{x}$	Obs.	Exp.	Z-score	$P > Z$
1.	BF	♀ (14)	8.93	121	280	−4.667	0.0001
	GB	♀ (25)	52.64	660	500		
2.	BF	♂ (28)	3.96	446	1,078	−6.808	0.0001
	GB	♂ (48)	32.67	2,480	1,848		
3.	BF	♀ (14)	8.93	356	301	1.477	0.1396
	BF	♂ (28)	3.96	547	602		
4.	GB	♀ (25)	52.64	1,222	925	3.448	0.0006
	GB	♂ (48)	32.67	1,479	1,776		

Results

Cosmocercoides variabilis was removed from the small and large intestines of 115 (39 females and 76 males) *Bufo a. americanus* in western West Virginia. All toads from Green Bottom (25 females and 48 males) were infected, but prevalences for Beech Fork toads were 87.5% (14 of 16 females) and 57.1% (28 of 49 males). Both female and male toads at Green Bottom had significantly lower body weights and significantly higher intensities of infection than their counterparts at the Beech Fork site (Tables 1, 2). Female hosts had higher intensities of infection than males at both collection sites, but the difference was significant only at Green Bottom (Table 2).

Of the 3,048 *Cosmocercoides variabilis* adults recovered during the course of this study, 1,852 were females, yielding a highly significant female-biased sex ratio of 1.55:1.00 (Table 3). This female-biased sex ratio was consistent in female and male hosts at the different collection sites, even though intensities of infection between host sexes and between sites were different (Table 3).

Discussion

High prevalences of *C. variabilis* observed in the present study (e.g., 100% in both sexes of Green Bottom toads; 87.5% and 57.1% for female and male toads, respectively, at Beech Fork) were not surprising. Vanderburgh and Anderson (1987b) reported high prevalences (e.g., 85% and 89% for late April and early May, respectively) for this nematode species in breeding populations of *B. a. americanus* from Ontario, whereas *C. dukae* (=*C. variabilis*?) was found in 75% of 24 *B. a. americanus* examined from central Ohio (J. C. McGraw, pers. comm.). Harwood (1930) reported *Oxysomatium variabilis* (=*C. variabilis*) in 38 of 44 (86.5%) *B. valliceps* from Texas.

Vanderburgh and Anderson (1987b) reported mean intensities of 3.5 and 3.8 for adult *C. variabilis* in late April and early May, respectively, and a mean intensity of 9.2 mature nematodes in summer and fall collections. Mean intensities of *C. variabilis* in our Beech Fork sample (Table 2) are not dissimilar to mean values reported in toads from Ontario. The considerably higher mean intensities in Green Bottom toads (Table 2) cannot easily be explained, although it is plausible that toads in populations where infection is high would be more likely to come in contact with more infective nematode larvae.

Data on parasitic infection by amphibian host sex do not often appear in the literature, and when they do, as Aho (1990) has pointed out, the influence of host gender on parasitic community structure is variable. His point is well taken. Mean intensities of *C. variabilis* infection

Table 3. Total numbers and sex ratios observed for *Cosmocercoides variabilis* in *Bufo a. americanus* populations from Beech Fork (BF) and Green Bottom (GB), West Virginia. ∞ = juveniles

Site	Host sex (*n*)	*Cosmocercoides variabilis* ♀♀	♂♂	∞	♀♀:♂♂	χ^2	*P*
BF	♀ (14)	70	40	15	1.75:1.00	8.18	<0.005
	♂ (28)	60	39	12	1.54:1.00	4.46	<0.05
GB	♀ (25)	786	513	17	1.53:1.00	57.37	<0.001
	♂ (48)	936	604	22	1.55:1.00	71.57	<0.001
Totals	(115)	1,852	1,196	66	1.55:1.00	141.19	<0.001

between female and male hosts at Beech Fork were not significantly different, whereas mean intensities were significantly different between sexes at Green Bottom (Table 2). Goldberg and Bursey (1991) reported different prevalences by gender in *Bufo punctatus* from Arizona, but no significant differences in prevalences by host gender were found in 3 toad species from New Mexico (Goldberg et al., 1995). There were no significant differences in prevalences by host sex in the present study at either Green Bottom (100% for both host sexes) or Beech Fork (87.5% and 57.1% for female and male toads, respectively; chi-square [Yates's correction] = 3.624, 1 df, $P = 0.0595$).

In warm-blooded hosts, the number of female nematodes typically exceeds that of males (Roche and Patrzek, 1966). Evaluation of nematode sex ratios in amphibian hosts has generally been ignored, although Muzzall (1990) and Joy et al. (1993) have provided some insights on this aspect of amphibian nematode biology. In the present study, statistically significant female-biased sex ratios of *C. variabilis* were quite consistent between host sexes, between collection sites, and even between hosts that showed significant differences in mean intensities (Table 3). This last point is of interest because Roche and Patrzek (1966) noted that female-biased sex ratios are "... often found in infections with a scanty number of worms."

In summary, our findings relative to prevalences and mean intensities corroborate those of other investigators, whereas our data on infections by sex of host and on nematode sex ratios are new. Still, the relationships between host sex and parasitic nematode infection rates in amphibians are not well understood. Future investigators, using appropriate sample sizes, could add appreciably to our knowledge of amphibian/nematode associations by segregating female from male hosts in their necropsy protocols and data analyses.

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Application to the International Commission on Zoological Nomenclature

Case 2932 *Haplotrema* Looss, 1899 (Digenea): proposed designation of *H. loossi* Price, 1934 as the type species

The purpose of this application is to designate the nominal species *Haplotrema loossi* Price, 1934, a spirorchid parasite of marine turtles, as the type species of the blood fluke genus *Haplotrema* Looss, 1899. At present the type species is *Distoma constrictum* Leared, 1862, but this is due to a misidentification and the genus was based on material later named *H. loossi.* The name *H. mistroides* (Monticelli, 1896) is a senior subjective synonym of *H. loossi.*

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